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ANTIGENIC DIFFERENCES BETWEEN CELLS OF A TRANSPLANTED STRAIN OF A  
HUMAN LEUKEMIA CULTURE (G-96) AND CELLS OF A COXSACKIE B  
VIRUS-RESISTANT CLONE OBTAINED FROM THIS CULTURE

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One of the principal factors of natural antiviral immunity is cell and tissue nonsusceptibility whose chief manifestation is the absence of virus multiplication in the tissues, the mechanisms of which are still unclear. Transplanted human leukemia cells (G-96) and clones obtained from this culture which are specifically nonsensitive to Coxsackie B virus [1] can serve as a convenient model for a study of these questions.

Data which are available at the present time on a comparative study of G-96 cells and resistant clones make it possible to assume that there evidently is in the cells of resistant clones genetically fixed reorganization of the cell metabolism which leads to the development of conditions which prevent virus reproduction [1]. A detailed study of the qualitative and quantitative differences in the composition of the cell proteins in the resistant and original cell cultures is of primary importance for determination of these conditions.

We have attempted to find differences in the composition of cell proteins with the help of immunological methods.

#### MATERIAL AND METHODS

The following immunological methods were used: the cytotoxic test, gel precipitation reaction (RPG), and the anaphylactic reaction with desensitization.

Cell suspensions of G-96 cells or cells of resistant clones grown on medium No. 199 with 10% bovine serum were used both for the anaphylactic reaction and for immunization of rabbits for the purpose of obtaining anticell sera. The cell suspensions were stored in a frozen state and were thawed and frozen three times



before use. Nucleoprotein fractions of G-96 and resistant clone cells obtained by L.N. Mikhedov by Belozerskiy's method served as the antigens in the RPG. The sera were obtained by immunization of rabbits according to the following scheme. First immunization cycle: one subcutaneous injection of antigen (suspension of 10 million cells) with Freynd's complete stimulant; antigen without stimulant was subcutaneously injected in the same dose after 2 weeks and after 1 more week; stimulatory bleeding from the aural vein (30 ml) was carried out after 1, 2 and 3 weeks. The second immunization cycle was carried out 3 weeks after the bleeding by means of one intramuscular injection of the same 10 million cells without stimulant with subsequent bleeding on the 7th-14th day. The antibody titer according to the cytotoxic test both on G-96 cells and on cells of resistant clones did not exceed 1:16. It decreased by 1-2 dilutions in the course of a month of storage at 4°.

The cytotoxic test was carried out by depositing 0.1 ml of the appropriate anticell serum in different dilutions on a single layer of G-96 or resistant clone cells grown in test tubes. The sera were not heated in order to retain the complement. The results of the cytopathic effect were read after 18-20 hours of the antiserum's action on the cells at 37°. Medium No. 199 with 2% bovine serum was used as the supporting medium.

The RPG was carried out in small Petri dishes using 1% Difco agar in physiological solution with a pH of 7.2. Holes drilled opposite each other in the agar were filled with the corresponding antigens and antisera. The plates were put in a humid desiccator at 37° for 18-20 hours after which bands of precipitation in the agar on the lines of contact of the antigen and antibodies appeared in transient light.

Guinea pigs weighing 320-350 g were used for the anaphylactic reaction with desensitization. Sensitization was accomplished by means of one subcutaneous injection of 20 million G-96 cells or the same amount of cells of one of the resistant clones. 20 million G-96 cells, like 20 million resistant clone cells, contain 1.6-1.7 mg of nitrogen according to the Kjeldahl method which in converting to protein is 10-11 mg. The main experiment which consisted of desensitization by means of the intravenous injection of 10 million cells and 20 million more cells after 2 hours was carried out after 30-35 days. In one of the experiments there was a 3rd desensitizing dose of 60 million cells. Animals in which a reaction to the last desensitizing dose was absent were injected after 2 hours with the resolving dose of antigen - 20 million cells. If the sensitization was carried out with resistant clones, the desensitization was carried out with G-96 and the resolving injection with resistant cells. If the sensitization was carried out with G-96 cells then the desensitization was conducted with cells of a resistant clone and the resolving injection with G-96 cells.

Selection of the cytotoxic test, RPG and the anaphylactic reaction with desensitization for demonstrating differences in the antigenic structure of the cells being compared depended on the following considerations. Since anticell antibodies mainly interact with antigens of the cell membrane in acting on whole



cells we can consider that in our experiments the cytotoxic test is directed mainly toward demonstrating differences between the membrane antigens of the G-96 cell and of the resistant clones. On the other hand, the nucleoprotein fraction of the cells used as the antigen in the RPG does not contain insoluble lipoproteins, that is, mainly membrane antigens and therefore provides the possibility of detecting a difference in the antigenic complexes located in internal structures of the cell.

The anaphylactic reaction with desensitization gives an idea of differences in the cells' whole antigenic complex and at the same time is the most sensitive reaction.

## RESULTS

Neither qualitative nor quantitative differences in the sera's cytopathic effect were found from the action of anticell serum to homologous cells and cells of a resistant clone on a single layer of a G-96 culture, just as from the effect of the same sera on a single layer of a resistant clone. In order to exclude possible individual characteristics of the sera, the following observations were also carried out: antiserum to a G-96 culture acted simultaneously on G-96 cells and cells of a resistant clone and in a parallel series antiserum to a culture of a resistant clone acted on the same 2 types of cells. Differences were also not found in this case. The optimal quantitative relations of the antigens from the cells being compared with the antisera homologous to them were determined first of all in the RPG. Clear lines of precipitation located midway between the holes filled with antigen and homologous antiserum both in experiments with a nucleoprotein fraction of G-96 cells and of cells of a resistant clone are obtained with the use of undiluted serum and the original concentration of the nucleoprotein fraction of the corresponding cells.

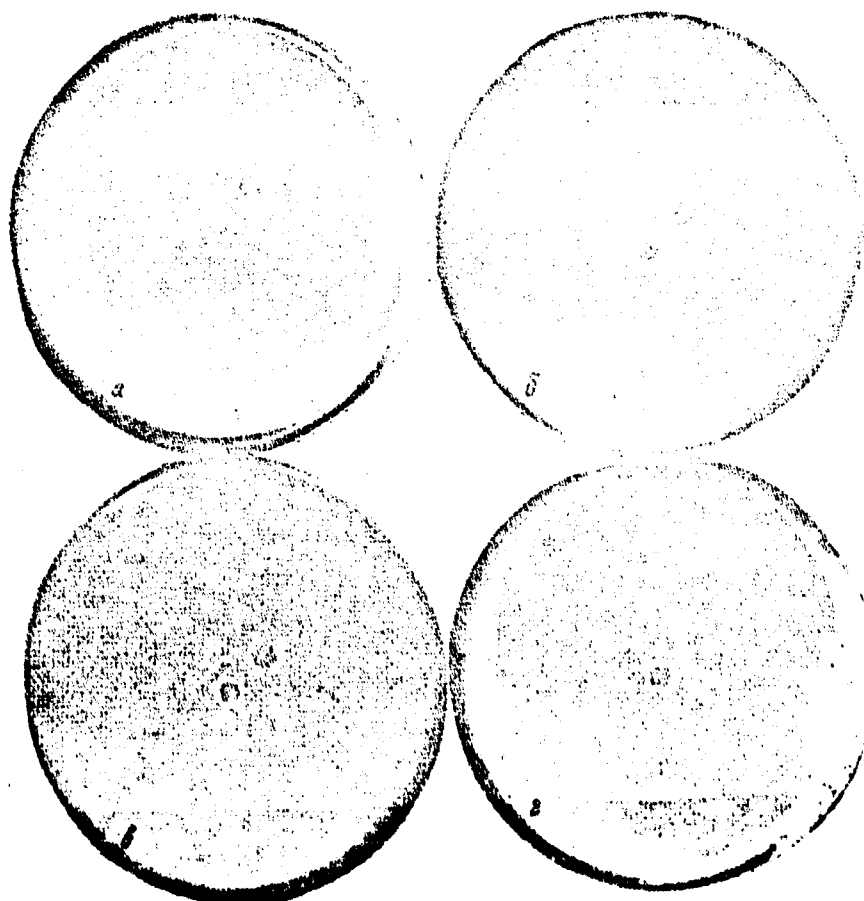
In setting up an RPG with antigen from G-96 cells and homologous antiserum and antiserum to a resistant clone, just as with antigen from resistant cells and the 2 sera named above, additional bands of precipitation indicating the presence of some additional antigen were not found, but a quantitative difference was clearly seen: clear lines of precipitation in the optimal zone with homologous antisera and very weak lines with heterologous. Moreover, the line of precipitation between the G-96 antigen and the antiserum to the resistant clone was strongly shifted toward the hole containing antiserum and between the antigen from a resistant clone and antiserum to G-96 cells the line of precipitation was also strongly shifted, but in the direction of the antigen. We shall also give a protocol of one of the experiments as an illustration (see figure).

The data presented evidently indicates quantitative changes among individual components of the antigenic complex in the nucleoprotein fraction of the resistant clone in comparison with G-96 cells. The absence of bands of precipitation in the RPG with antigen from G-96 cells and antiserum to the same cells which were first depleted by an excess of cells of a resistant clone indicates that the antigenic differences between G-96 cells



and the cells of resistant clones are not connected with the appearance of some new antigen or with the disappearance of an antigen which was there previously, but with changes in the quantitative relations of individual components in the cells' antigenic complex. Thus, for depletion of the antiserum to G-96 cells, it was necessary to saturate it with homologous antigen in a ratio of 1:1, while for depletion of the same serum with antigen from resistant cells 3 times more of the latter was required, that is, the ratio of serum and antigen was 1:3. Antiserum to a resistant clone was also devoid of the capacity to produce a band of precipitation with its antigen after preliminary depletion with G-96 cells.

The results of searches for antigenic differences between G-96 cells and cells of resistant clones according to data of the anaphylactic reaction with desensitization are presented in the table. As is seen from the table, if guinea pigs sensitized with G-96 cells were desensitized with cells of a resistant clone



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Precipitation reaction in gel. a) Central hole G-96 antigen, side holes antiserum to G-96 cells; b) central hole antigen of Clone 12, side holes antiserum to Clone 12; c) central hole G-96 antigen, left hole antiserum to G-96 cells, right hole antiserum to Clone 12; d) central hole antigen of Clone 12, left hole antiserum to G-96 cells, right hole antiserum to Clone 12.



TABLE 495-A

Antigenic Differences Between G-96 Cells and Cells of Resistant Clones According to Data of Anaphylactic Reaction With Desensitization

Сенсибилизация 1	Последняя десенсибилизация 2	Разрешающее введение 1-й сенсибилизирующей дозы клеток 3				
		отсутствие реакции 4	+	++	+++	всего с наличием реакции 5
Клетками G-96 6	Резистентными клетками в дозе, равной одной в сенсибилизирующей	0/10	3/10	4/10	3/10	10/10
	Резистентными клетками в дозе, равной трем в сенсибилизирующей	7/7	0/7	0/7	0/7	0/7
7 Клетками резистентного клона	10 Клетками G-96 в дозе, равной одной сенсибилизирующей	4/4	0/4	0/4	0/4	0/4

Notation: + decrease in temperature, scratching; ++ decrease in temperature, scratching, sneezing, coughing, pulling of hair; +++ severe shock.

1) Sensitization; 2) last desensitization; 3) resolving injection of 1st sensitizing dose of cells; 4) absence of reaction; 5) total with presence of reaction; 6) with G-96 cells; 7) with cells of a resistant clone; 8) with resistant cells in a dose equal to one sensitizing dose; 9) with resistant cells in a dose equal to three sensitizing doses; 10) with G-96 cells in a dose equal to one sensitizing dose.

and the last desensitization was carried out with a dose of cells equal to the sensitizing dose, then an anaphylactic reaction was observed in all the experimental animals from a resolving injection of G-96 cells.

If the sensitization was carried out with resistant cells and the desensitization with G-96 cells, then a reaction was not observed from a resolving injection of resistant cells. These experiments, it would seem, spoke of the presence of antigenic differences: some antigen which is presented in a G-96 culture is absent from a resistant clone. But, as we succeeded in establishing, these antigenic differences are only of a quantitative character. If guinea pigs sensitized with G-96 cells were desensitized with a triple amount of resistant cells, an anaphylactic reaction was not observed. Thus, the results of the anaphylactic reaction with desensitization agree with the results of the RPG. It is necessary once again to draw attention to the fact that the antigen for the RPG (soluble nucleoprotein fraction) reflects mainly the antigenic composition of internal cellular structures.



The data presented, in our opinion, make it possible to assume that one of the mechanisms leading to the transformation of a susceptible cell into a nonsusceptible can be quantitative changes in the cells' antigenic complex in which a relative decrease in the concentration of individual cellular antigens occurs.

#### CONCLUSIONS

1. From a crossing over effect on G-96 cells and on cells of a clone resistant to Coxsackie B virus by the anticell sera corresponding to them differences in the cytopathic effect of these sera were not found.

2. In a cross precipitation reaction in gel with antigens from sensitive or resistant cells and with the antisera corresponding to them additional bands of precipitation indicating the presence of some additional antigen were not observed, but a quantitative difference in the composition of the antigenic complexes of G-96 cells and cells of a resistant clone is clearly demonstrated.

3. The presence of a quantitative difference in the composition of the antigens in G-96 cells and cells of a resistant clone was also established with the help of the anaphylactic reaction with sensitization.

#### REFERENCES

1. Solov'yev, V.D., Vopr. virusol., 1964, No. 5, page 580.

#### ANTIGENIC DIFFERENCES BETWEEN CELLS OF A CONTINUOUS STRAIN OF HUMAN LEUKEMIA CULTURE (G-96) AND CELLS OF COXSACKIE B VIRUS-RESISTANT CLONE DERIVED FROM THIS CULTURE

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#### SUMMARY

By means of cytotoxic test, gel precipitation test and anaphylaxy test with desensitization it was shown that antigenic differences between the cultures under study consist in different quantities of individual components in the antigenic complex of the cells.